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Interictal serum brain-derived neurotrophic factor level reflects white matter integrity, epilepsy severity, and cognitive dysfunction in chronic temporal lobe epilepsy

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ABSTRACT

Objective: Most patients with temporal lobe epilepsy (TLE) have epileptic foci originating from the medial temporal lobe, particularly the hippocampus. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin growth factor mainly expressed in the hippocampus, though it is not known whether the circulating level of BDNF reflects cognitive performance or white matter structural changes in chronic TLE.
Methods: Thirty-four patients with TLE and 22 healthy controls were enrolled for standardized cognitive tests, diffusion tensor imaging, and serum BDNF measurement. The patients were further divided into a subgroup with unilateral TLE ($n = 23$) and a subgroup with bilateral TLE ($n = 11$) for clinical and neuroimaging comparisons.
Results: There were significantly lower BDNF levels in the patients with TLE compared with the controls, with significance contributed mainly from the subgroup with bilateral TLE, which also had more frequent seizures. The BDNF levels correlated with epilepsy duration ($\sigma = -0.355$; $p = 0.040$) and fractional anisotropy (FA) in the left temporal lobe, left thalamus, and right hippocampus. Using a regression model, BDNF level predicted verbal memory score. Further, design fluency scores were predicted by serum BDNF level via the interactions with left temporal FA.
Conclusions: Serum BDNF levels reflected longer epilepsy duration, impaired white matter integrity, and poor cognitive function in patients with chronic TLE.

1. Introduction

With an estimated prevalence of 40–60% in adult epilepsy, temporal lobe epilepsy (TLE) remains to be a common epilepsy syndrome that is often refractory to antiepileptic drugs [1,2]. Although the seizure focus in most cases of TLE originates from the medial temporal lobe, particularly the hippocampus, virtually all patients would develop complex partial seizures with or without secondary generalization [3]. Temporal lobe epilepsy can be a progressive disorder, potentially resulting in cognitive deficits over time [4]. Impairment in cognitive performance beyond the memory domain has been frequently reported in patients with TLE [5,6]. In the chronic phase, structural atrophy in the extratemporal regions was found to relate to cognitive deficits [7].

Patients with TLE can be categorized into two clinical phenotypes by interictal epileptiform discharges, subgroups with unilateral TLE and bilateral TLE [8–11]. Thirty-five to 61% of patients belong to the subgroup with bilateral TLE. Although simplistic, the electroencephalogram classification provides prognostic value. The subgroup with unilateral TLE often demonstrates better surgical outcomes compared with the subgroup with bilateral TLE [11,12]. In addition, the subgroup with bilateral TLE is often more refractory to antiepileptic drug (AED) treatment.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors mainly expressed in the hippocampus network. It has been demonstrated that BDNF can cross the blood–brain barrier in two directions, from the brain to the peripheral blood and from the blood to the brain via the high capacity saturable transporter system [13]. The high correlation between cortical and serum BDNF [14,15] has led to a number of human studies on BDNF levels in various neurological diseases. In addition to the central nervous

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system, there are other potential peripheral sources that may affect BDNF serum levels. These include secretion from platelets [16], vascular endothelial cells [17], monocytes during activation [18], or myocytes [19]. In the enteric nervous system, visceral epithelia are a major source of BDNF and play a role in modulating enteric neuronal activity and synaptic communication [20].

In surgically resected hippocampus, increased levels of BDNF mRNA and protein have been found, indicating that epileptic activity may upregulate the protein level via *BDNF* gene expression [21,22]. However, a decrease of serum BDNF level has also been reported in adult patients with epilepsy [23]. Other factors, such as oxidative stress, blood–brain barrier damage, deregulated neuroinflammation, and the nonfasting state, have been related to changes in serum BDNF level [24–26]. Most studies have focused on BDNF levels and their relationships with seizures or epilepsy syndromes as compared to controls; there is a paucity of literature evaluating cognitive performance and BDNF changes in patients with chronic TLE.

As the brain is organized into segregated networks, large-scale functional connectivity can be altered if the major fiber tracts are disrupted by repeated epileptic discharges. Recent diffusion tensor imaging techniques provide in vivo quantification of WM integrity by measuring fractional anisotropy (FA) values [27]. These techniques are believed to assess such factors such as myelination, axonal density, and/or integrity [28]. The development of a white matter (WM) parcellation algorithm allows approximating the 3D trajectories of major WM bundles by probabilistic maps [29]. With the automated tract-specific quantification of FA, the relationships of serological BDNF and related tract integrity can be modeled.

The present study aimed to investigate the prognostic value of serum BDNF level and its correlation with cognitive function or neuroimaging parameters in patients with chronic TLE. Based on the literature search, we hypothesized that patients with a more severe clinical status, i.e., the subgroup with bilateral TLE, may show lower serum BDNF levels and worse cognitive performances. The clinical significance of serum BDNF level on cognitive performance can be verified by the changes of neuroimaging parameters.

2. Methods

2.1. Study design

This was a single center, age- and sex-matched case–control study which was approved by the Institutional Review Board of Chang Gung Memorial Hospital and complied with the ethical standards established in the Declaration of Helsinki. The experiments were undertaken with the written, informed consent of each subject and their caregiver (when appropriate).

2.2. Patient enrollment

This study was initiated at the epilepsy outpatient clinic of the Kaohsiung Chang Gung Memorial Hospital in 2009. All underwent an extensive investigation including clinical history, comprehensive neurologic examination, and routine visual MRI analysis [7]. In this study, we only enrolled those with electroencephalography (EEG) showing interictal activity.

The clinical diagnosis of TLE was based on the International League Against Epilepsy criteria (1997) as follows: (1) seizure semiology consistent with TLE, with abdominal, epigastric, psychic, or autonomic auras, followed by behavioral arrest, progressive alteration of consciousness, oroalimentary, and manual automatisms; (2) mesial and/or anterior temporal interictal spikes from video-electroencephalography (EEG) or bilateral sphenoidal EEG; and (3) no lesions other than increased T2 signal and/or atrophy in hippocampal formation identified by MRI.

Because it was not possible to combine all the influential factors in the group with TLE to produce a uniform population, we only included nonsurgical patients. By family history and past medical history review, none of the patients selected in this study had a family history of epilepsy or a childhood febrile seizure history. Additional exclusion criteria in this study included a known history of mental retardation and a psychiatric comorbidity that prevented either a neuropsychiatric interview or neuroimaging. We also excluded patients with any of the following: (1) medication history of psychoactive or central nervous system depressant drugs and (2) abnormal liver or renal functions. These exclusion criteria were added to avoid the confounding effects of medication and physical disorders on the cognitive test results.

After screening our cohort with TLE [30], 34 patients fulfilled the inclusion and exclusion criteria, agreed to participate in the study, and completed it. The age at onset, duration of epilepsy, average seizure frequency per month during the previous year, and numbers of AEDs were analyzed. According to the interictal discharges on EEG [8,11], the patients were further divided into two groups (subgroup with unilateral TLE [n = 23; 11 left and 12 right temporal] and subgroup with bilateral TLE [n = 11]). Twenty-two age- and sex-matched healthy controls from the normative database were used for BDNF level, neuropsychological testing [31], and MRI comparisons [32].

2.3. Cognitive and behavior testing

All of the cognitive test protocols [33] were performed 2 h before the MRI during the interictal state for further subgrouping. General intellectual function was assessed using the Mini-Mental State Examination (MMSE). Verbal and nonverbal episodic memory was assessed by the Chinese Version Verbal Learning Test (CVVLT) [31] and the Rey–Osterrieth Complex Figure after a 10-minute delay. Speech and language abilities were measured with the 15-item Boston Naming Test and semantic verbal fluency. Visuospatial abilities were assessed by a modified Rey–Osterrieth Complex Figure. Executive function [34] was assessed by digit backward span, design fluency, Stroop Interference test, and the Modified Trails B test.

2.4. BDNF analysis

As discussed in the literature, a number of determinants may influence the BDNF levels. There is a diurnal variation for plasma BDNF levels but not in for serum levels [35,36]. Therefore, we measured the serum level in this study. In addition, a nonfasting state is related to an attenuated BDNF level [26]. As we hypothesized that patients with more severe clinical status may show lower serum BDNF levels, we decided to unify the time line to a fasting state in the study population to avoid the possible floor effect of BDNF levels in the patients. Meanwhile, to explore the impacts related to epilepsy or seizure severity and to avoid possible demographic confounders, we also matched the subjects for gender and age, time of sampling in the cooled box (<30 min), and duration of sample storage (<1 week).

The time intervals between blood sampling and the last seizure and last secondary generalized tonic–clonic seizure ranged from 2 to 700 days. Blood samples were taken between 8 and 10 am after overnight fasting for BDNF analysis. Blood for serum BDNF was collected in anticoagulant-free tubes with the clotting activator and kept for 1 h on ice with a temperature of about 4 °C. Serum BDNF levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (BDNF E_{max}® ImmunoAssay System Technical Bulletin). The degrees of enzymatic turnover of the substrate were determined by dual wavelength absorbance measurements at 450 nm using a multiscan spectrum reader (Thermo Scientific, Miami, FL, USA). The antigen standards were used to plot a standard curve of absorbance versus antigen concentration from which the antigen concentrations in the unknowns were calculated. The intra- and inter-assay variations were less than 9 and 15%, respectively.